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Title

Long-term oxytocin administration enhances the experience of attachment

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Highlights

- We examined effects of multiple-dose intranasal oxytocin (OT) on attachment
- State and Trait attachment were assessed before and after two-week OT administration
- OT treatment decreased attachment avoidance and increased attachment toward peers
- Participants with less secure peer attachment showed most pronounced OT effects
- OT treatment was also associated with changes in mood

ABSTRACT

The neuropeptide 'oxytocin' (OT) is known to play a pivotal role in a variety of complex social behaviors by promoting a prosocial attitude and interpersonal bonding. Previous studies showed that a single-dose of exogenously administered OT can affect trust and feelings of attachment insecurity. With the present study, we explored the effects of two weeks of daily OT administration on measures of state and trait attachment using a double-blind between-subjects randomized placebo-controlled design. In 40 healthy young adult men state and trait attachment were assessed before and after two weeks of daily intranasal OT (24 IU) or placebo using the State Adult Attachment Scale and the Inventory of Parent and Peer Attachment. Mood, social responsiveness and quality of life were additionally assessed as secondary outcome measures. Reductions in attachment avoidance and increases in reports of attachment toward peers were reported after two weeks of OT treatment. Further, treatment-induced changes were most pronounced for participants with less secure attachment towards their peers. indicating that normal variance at baseline modulated treatment response. OT treatment was additionally associated with changes in mood, indicating decreases in feelings of tension and (tentatively) anger in the OT group, not in the placebo group. Further, at the end of the two-week trial, both treatment groups (OT, placebo) reported to experience an increase in social responsiveness and quality of life, but the effects were only specific to the OT-treatment in terms of reports on 'social motivation'. In summary, the observed improvements on state and trait dimensions of attachment after a multiple-dose treatment with OT provide further evidence in support of a pivotal role of OT in promoting the experience of attachment.

KEYWORDS

Oxytocin, State attachment, Trait attachment, Mood, Social responsiveness, Quality of life

1. Introduction

The neuropeptide 'oxytocin' (OT) is known to play a pivotal role in a variety of complex social behaviors. OT is a nonapeptide produced by the hypothalamic paraventricular and supraoptic nuclei and is secreted into the bloodstream by the posterior pituitary gland. Based on initial animal and human research, the physiological role of OT in lactation and childbirth is well-established (Galbally et al., 2011; Insel, 2010; Kendrick, 2000; Sue Carter, 1998). More recently, different lines of research have shown a strong involvement of OT in complex social behaviors including interpersonal bonding, maternal care, and the ability to establish trust and form social attachments (for reviews see Bakermans-Kranenburg and van IJzendoorn (2013), Bartz et al. (2011), Guastella and MacLeod (2012), but also Nave et al. (2015)).

From **genetic research**, important insights on the involvement of the oxytocinergic system in pair-bonding and attachment have emerged. For example, recent research showed that genetic variations in the OT-receptor gene (OXTR) of *prairie voles* are related to social attachment and partner preference (King et al., 2015), and that knock-down of the OT receptor inhibited social attachment and parental care (Keebaugh et al., 2015). Also in *human infants*, polymorphisms in the OXTR gene have been associated with variations in attachment security (Chen et al., 2011).

Associations have also been shown between **endogenous levels of OT** in plasma and maternal or paternal bonding behaviors, attachment-related thoughts and infant social engagement in naturalistic settings (Feldman et al., 2010, 2007; Gordon et al., 2010). However, studies investigating a link between plasma OT levels and trust behavior in experimental settings (trust game paradigms), have yielded mixed results with some studies reporting tentative links (Zak et al., 2005; Zhong et al., 2012), while others failed to reveal a significant correlation (Christensen et al., 2014).

Other evidence for a role of OT in the establishment of trust and attachment came from studies investigating the effects of **exogenously administered OT** on behavior and neural functioning. For example, seminal work by Kosfeld et al. (2005) showed that intranasally administered OT can increase trust among human individuals. Particularly, using a social trust game with monetary stakes, Kosfeld et al. (2005) showed that a single-dose of OT significantly increased the readiness to bear social risks arising through interpersonal interactions. Later, Buchheim et al. (2009) showed that OT can increase the experience of attachment security, while De Dreu (2012) proved that intranasal OT can facilitate the development of trust and cooperation in particular in adults with high attachment avoidance (by reducing betrayal aversion). Also self-reports on agency towards self or others were shown to be influenced by single doses of OT administration, indicating that in avoidantly attached individuals, OT positively influenced communal traits and agency towards others (Bartz et al., 2015).

Interestingly, subsequent **neuroimaging work** provided indications that the effects of exogenously administered OT on trust and trust adaptation were associated with reductions in neural activity of brain regions that are implicated in fear processing (amygdala and midbrain regions) (Baumgartner et al., 2008).

Together, these aforementioned studies provide promising indications that a single-dose of exogenously administered OT can affect trust and attachment behavior in humans. To extend this line of work, the present study aimed to provide an initial investigation on the effects of multiple-dose treatments with OT on measures of state and trait attachment. To do so, we conducted a double-blind between-subjects randomized placebo-controlled trial assessing the effects of two weeks of daily OT administration using the State Adult Attachment Measure (SAAM) (Gillath et al., 2009) and the Inventory of Parent and Peer Attachment (IPPA) (Armsden and Greenberg, 1987) as primary outcome measures. The IPPA is constructed to measure perception of secure attachment towards peers, parents and significant others at a trait level. The SAAM on the other hand, is constructed to measure transient changes in attachment anxiety, attachment avoidance and attachment security at a state level.

To explore whether changes after multiple-dose OT intake were potentially related to changes in mood, we additionally assessed changes in mood states using the Profile of Mood States questionnaire (POMS) (McNair and Lorr, 1964). Additional secondary outcome measures were included to obtain an assessment of social responsiveness and general reports of quality of life. To this end, the adult self-report version of the Social Responsiveness Scale (SRS) (Constantino and Gruber, 2005) and the abbreviated version of the World Health Organization Quality of Life questionnaire (WHOQOL) (WHO, 1998) were used, respectively.

2. Materials and methods

2.1. Study design

The study design involved a randomized, double-blind, placebo-controlled, between-subjects trial to assess multiple-dose effects of intranasal oxytocin (OT) administration. Written informed consent was obtained from all participants prior to the study. Consent forms and study design were approved by the local Ethics Committee for Biomedical Research at the University of Leuven, KU Leuven (S56327) in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki). The trial was registered with the European Clinical Trial Registry (Eudract 2014-000586-45) and the Belgian Federal Agency for Medicines and Health products.

2.2. Participants

A total of 40 healthy young adult males were included in the study. Participants were randomly allocated to receive OT or placebo (PL) nasal sprays (20 OT, mean age = 20.70, S.D. = 2.72; 20 PL, mean age = 21.55, S.D. = 2.39). All participants were right-handed (self-reported) and mean age did not differ between OT and PL groups.
All participants were recruited through advertisement within the university, such that 90% of the included sample were university students. Only male participants were recruited to avoid sex differences in OT response or potential interactions with the female hormonal cycle. Exclusion criteria were (i) age below 18 or above 30 years old (ii) a diagnosed psychiatric or neurological disorder, (iii) intake of psychotropic medication, (iv) history of neurological disease, and (v) history or evidence of other diseases (cancer, hematologic illness, endocrine disease, cardiovascular disease, respiratory condition, renal disease, liver condition or gastrointestinal illness).

2.3. Drug protocol

Sprays were prepared by the KU Leuven University Hospital pharmacist. OT (Syntocinon®, Sigma-tau) and placebo (PL) (saline natrium-chloride solution) were administered in amber 15 ml glass bottles with metered pump (ACA Pharma). Each puff per nostril contained 4 international units (IU) of OT. Each participant received a total of 14 doses of 24 IU, delivered daily over 14 consecutive days. All participants received clear instructions about the use of the nasal spray. At first use, air present in the nasal spray was removed by pumping the spray until a fine mist was observed. Participants were instructed to keep one nostril closed, to take a

deep breath through the nose and to tilt their head slightly backwards during nasal administration in order to minimize gravitational loss of the spray. To assure proper use of the spray and to validate tolerability, each subject administered the first dose in front of the experimenter and commented on their experience (e.g. particular smell or taste). All participants were monitored onsite until approximately one hour after nasal spray administration. Participants were asked to take the nasal spray in the morning; and to keep a daily record of the time point of nasal spray administration and whether or not they were alone or in company of others the first two hours after administration (records were not returned by 2 OT participants and 2 PL participants). Percentage of days at which the spray was administered in the presence of others was not significantly different between treatment groups (OT: 75.6 % (SD 23.8); PL: 69.8 % (SD 28.6); $t(34)= .32$; $p = .75$).

All participants were screened for potential adverse events or side effects. The side effects questionnaire and a frequency table of reported side effects are provided as Supplementary Tables 1 and 2. As listed in more detail in Supplementary Table 2, only minimal side effects were reported and effects were independent of treatment (e.g., participants from both treatment groups reported to feel more focused (2 OT; 2 PL) or confident (1 OT; 1 PL)). Finally, at the end of the trial, participants were asked if they thought they had received OT or PL. Only two participants correctly guessed to have received the OT treatment. All other participants thought they had received PL.

2.4. Procedure

Self-report questionnaires on attachment were used as primary outcome measures assessing the effects of two weeks of daily OT administration. Additionally, questionnaires on mood, social responsiveness and quality of life were used as secondary outcome measures.

2.5. Questionnaires

2.5.1. State Adult Attachment Measure (SAAM)

The SAAM is a questionnaire to assess temporary fluctuations in state attachment (Gillath et al., 2009). The questionnaire contains 21 statements where participants have to indicate their current state (‘right now’) on a seven-point Likert-scale. The questionnaire comprises three subscales assessing (i) attachment security (e.g., *“I feel like I have someone to rely on”*) (7 items; α = .82-.91); (ii) attachment anxiety (e.g., *“I feel a strong need to be unconditionally loved right now”*) (7 items; α = .81-.85); and (iii) attachment avoidance (e.g., *“If someone tried to get close to me, I would try to keep my distance”*) (7 items; α = .71-.87) (Gillath et al., 2009).

2.5.2. Inventory of Parent and Peer Attachment (IPPA)

The IPPA is a questionnaire to assess trait attachment to (i) mother (25 items; α = .87); (ii) father (25 items; α = .89); (iii) peers (25 items; α = .92); and (iv) an important person of choice (25 items; no reported α) (Armsden and Greenberg, 1987). The IPPA questionnaire comprises a total of 100 items (25 for each section) with a four point Likert-scale to assess three dimensions of attachment: degree of mutual trust, quality of communication and extent of anger and alienation. The fourth part (attachment to an important person) was not mandatory and was only obtained from 11 OT and 16 PL participants (50% partner, 21.87% close friend, 28.13% unanswered). One participant of the OT group did not complete the section on 'father'.

2.5.3. Profile of Mood States (POMS)

A 32-item short version of the POMS questionnaire (McNair and Lorr, 1964; Wald and Mellenbergh, 1990) was used as a measure of transient affective states in order to assess whether mood levels of participants changed over the course of the study. This instrument comprises emotional adjectives subdivided in five domains: 'tension' (6 items; α = .84), 'depression' (8 items; α = .91), 'vigor' (5 items; α = .81), 'fatigue' (6 items; α = .90) and 'anger' (7 items; α = .87) which have to be rated on a five-point Likert scale. Participants were asked to rate their current mood state ('right now').

2.5.4. Social Responsiveness Scale (SRS) – Adult version

The Dutch adult self-report version of the SRS (Constantino, 2005; Roeyers et al., 2011) was adopted in our study to assess social responsiveness at baseline and post OT-treatment. The SRS (64 items) assesses variations in social responsiveness in the typical population and autism spectrum disorders using a four-point Likert-scale. It encompasses four subscales, including social communication (22 items; α = .88), social awareness (19 items; α = .80), social motivation (11 items; α = .83) and rigidity/repetitiveness (12 items; α = .79). Higher scores indicate less social responsiveness.

2.5.5. World Health Organization Quality of Life (WHOQOL) - Bref

The abbreviated version of the WHOQOL is a 26-item questionnaire to assess general quality of life related to physical health, psychological health, social relationships, and environment (α = .84) (WHO, 1998). Ratings vary from 1 (very bad, very unsatisfied, totally not or never) to 5 (very good, very satisfied, totally or always).

2.6. Data analysis and statistics

For each questionnaire, total scores and/or subscale scores were calculated. Shapiro-Wilk's W tests were used to investigate the normality of data distribution. At the group level, extreme

outliers were identified when scores were larger or smaller than Q3 ± 3(Q3-Q1), with Q1 and Q3 being the first and third quartile (Statistica 10; StatSoft. Inc. Tulsa, USA). No extreme outlier scores were identified in the distribution of the pre- and post-treatment scores for any of the questionnaires. However, a few extreme outliers were identified in the change-from-baseline scores (IPPA peer attachment: 1 PL outlier; IPPA father attachment: 1 PL outlier; SRS total: 2 OT outliers; POMS depression: 1 PL and 2 OT outliers; POMS angry: 2 OT outliers). For completeness, all primary statistical analyses are reported with and without exclusion of these outliers. Particularly, for all questionnaires, repeated-measures Analyses of Variance (ANOVA) were conducted with the between-subject factor 'group' (OT and PL) and the within-subject factor 'time' (Baseline, Post). Further, to assess the effect size of the OT treatment, Cohen's d (Cohen, 1988) was calculated by subtracting the change-from-baseline scores of the PL group from the change-from-baseline scores of the OT group [(Score change$_{OT}$ – Score change$_{PL}$)/ sqrt(SD$_{OT}$^2 + SD$_{PL}$^2)]. All statistics were executed with Statistica 10 (StatSoft. Inc. Tulsa, USA). The significance level was set at $p < .05$ for all analyses.

3. Results

Questionnaire scores are listed separately for each treatment group (OT, PL) and session (Baseline, Post) in Supplementary Table 3. Table 1 lists the change-from-baseline scores for each treatment group and the corresponding Cohen's d effect sizes.

3.1. Primary outcome measures: Trait and state attachment

3.1.1. Trait attachment: Inventory of Parent and Peer Attachment (IPPA)

Two-way ANOVA analysis revealed a significant 'group x time' interaction (with outlier: $F(1,38) = 6.49$, $p < .05$; $\eta^2 = .15$; power = .70; without outlier: $F(1,37) = 8.02$, $p < .01$, $\eta^2 = .18$; power = .79) for the subscale score 'Friends', indicating an increase in self-reported trait attachment towards peers in the OT group ($F(1,38) = 6.11$, $p < .05$), not in the PL group (with outlier: $F(1,38) = 1.28$, $p = .27$; without outlier: $F(1,37) = 0.00$, $p = 1.0$) (Cohen's d = .75, medium to large effect (with outlier); d = .64, medium effect (without outlier)) (Figure 1A (upper graph) and Table 1). Note that baseline scores in peer attachment were not significantly different between treatment groups ($F(1,38) = .01$, $p = .94$).

For the reports of trait attachment towards 'Mother' and 'Father', scores tended to increase after two weeks of OT treatment, but the change failed to reach significance (Mother: $F(1,38) = 1.97$; $p = .17$; $\eta^2 = .05$; power = .28) (Father: with outlier: $F(1,37) = 1.26$; $p = .03$; $\eta^2 = .05$, power = .20; without outlier: $F(1,36) = .34$; $p = .56$; $\eta^2 = .01$, power = .09). Reports of attachment towards an important person were only completed by a subset of participants (11 OT, 16 PL) and no significant treatment-related changes were revealed for this subscale ($F(1,25) = 0.09$,

p = .77; η^2 = .003; power = .06). Across treatment groups, baseline reports on attachment towards peers, mother and father were significantly inter-correlated (all, r > .62; p < .001).

To explore whether inter-individual differences in baseline IPPA peer attachment scores were related to treatment-related effects on post-treatment peer attachment scores, a step-wise multiple regression analysis was conducted covarying for baseline scores and testing baseline-by-treatment interactions (Supplementary Table 4 and Figure 1A). Analyses showed that differences in post-treatment IPPA scores between the OT and PL treatment groups were only evident after correction for variance in baseline scores (treatment effect without correction for baseline scores: β = .17, t(37) = 1.05, p = .30) (treatment effect with correction for baseline scores: β = .15, t(36) = 3.01, p < .01). Importantly, also a significant 'treatment group x baseline score' interaction effect was revealed (β = -.64, t(35) = -2.17, p < .05), indicating that the treatment effect differed according to initial (baseline) severity. Particularly, as visualized in figure 1A (lower graph), the shape of the interaction pattern indicated that the difference in outcome between treatment groups increased with increasing baseline 'severity' (lower baseline peer attachment). In other words, the OT treatment appeared to enhance IPPA peer attachment particularly among those with low peer attachment at baseline.

3.1.2. State attachment: State Adult Attachment Measure (SAAM)

A significant 'group x time' interaction effect was revealed for the avoidance subscale (F(1,38) = 4.36, p < .05; η^2 = .10; power = .53), indicating that state attachment avoidance significantly decreased after the two-week treatment in the OT group (F(1,38) = 5.35, p < .05), but not in the PL group (F(1,38) = .41, p = .53) (Cohen's d = .63, medium effect) (Figure 1B (upper graph) and Table 1). Baseline scores in attachment avoidance were not significantly different between treatment groups (F(1,38) = .10, p = .77). No significant 'group x time' interaction effects were revealed for reports of 'attachment security' (F(1,38) = .50, p = .49; η^2 = .01; power = .11) and 'attachment anxiety' (F(1,38) = .001, p < .97; η^2 < .001; power = .05), indicating that the two-week OT treatment did not exert a significant effect on these subscales.

To explore whether inter-individual differences in baseline SAAM attachment avoidance were related to treatment-related effects on post-treatment attachment avoidance scores, a step-wise multiple regression analysis was conducted covarying for baseline scores and testing baseline-by-treatment interactions (Supplementary Table 4 and Figure 1B). Analyses showed a significant difference in post-treatment attachment avoidance scores between the OT and PL treatment groups irrespective of variance at baseline (treatment effect without correction for baseline scores: β = -.32, t(38) = -2.07, p < .05) (treatment effect with correction for baseline scores: β = -.28, t(37) = -3.21, p < .01). Although note that the treatment effect was more pronounced after removal of variance explained by the baseline scores. No significant 'treatment group x baseline score' interaction effect was revealed (β = -.08, t(36) = -.35, p =

.73), indicating that the main effect of treatment (the vertical distance between the regression lines in figure 1B) was constant irrespective of initial baseline score. Note however that for both groups (OT and PL), reductions in SAAM attachment avoidance were most pronounced for participants with high baseline attachment avoidance.

3.1.3. Relationship between OT-related improvements in state (SAAM) and trait (IPPA) attachment

At baseline, significant inter-correlations were revealed between reports on peer/parent attachment (IPPA) and attachment security (SAAM) (all, $r > .50$; $p < .05$), whereas no significant relationships were revealed with baseline reports of attachment anxiety or avoidance (SAAM) ($p > .05$). Also no significant correlations were revealed between OT-related improvements in SAAM-based attachment avoidance and IPPA-based improvements in peer attachment (OT-group: $r = -.03$; $p = .92$). This finding indicates that high improvements on state attachment avoidance were not necessarily predictive of high improvements in secure peer attachment (although as a group, participants receiving OT improved on both measures).

3.1.4. Treatment-related changes in mood: Profile of Mood States (POMS)

Compared to the PL group, participants receiving OT reported to feel significantly less tense ('group x time' interaction: $F(1,38) = 4.29$, $p < .05$; $\eta^2 = .10$; power = .52) and less angry ('group x time' interaction: $F(1,38) = 4.37$, $p < .05$; $\eta^2 = .10$; power = .53) after two weeks of nasal spray treatment (Figure 2), but note that the effect on angry mood states may have been driven by an outlier subject (i.e., no significant effect after removal of the outliers: $F(1,36) = 2.03$, $p = .16$; $\eta^2 = .05$, power = .28). No significant effects were found for the other mood states (depression: with outliers: $F(1,38) = 1.3$, $p = .25$; $\eta^2 = .03$; power = .21; without outliers: $F(1,35) = .00$, $p = 1.00$, $\eta^2 = .00$, power = .05) (vigor: $F(1,38) = .15$, $p = .70$; $\eta^2 = .003$; power = .06) (fatigue: $F(1,38) = .21$, $p = .65$; $\eta^2 = .006$; power = .07).

To explore whether inter-individual differences in OT-related improvements in attachment were related to inter-individual differences in mood changes, a general linear regression analysis was conducted with 'change in mood' as a covariate-of-no-interest. Correlation analysis revealed that treatment-related decreases in attachment avoidance (SAAM) were positively correlated with decreases in reports of anger (with outlier: $r = .52$; $p < .05$), but not tension ($r = .22$; $p = .33$), but note that the effect of angry mood states may again have been driven by an outlier subject (i.e., no significant effect after removal of the outliers: without outlier: $r = .23$, $p = .36$). Regression analysis further confirmed that the response to OT on attachment avoidance was - at least partly – accounted for by more basic changes in mood. Particularly, the OT-dependent improvements in attachment avoidance only tentatively persisted after correction for changes in 'tension' ($F(1,37) = 2.87$; $p = .09$) and no longer reached significance

after correction for changes in 'anger' ($F(1,37) = 2.06$; $p = .15$). On the other hand, for reports on changes in peer attachment (IPPA), correlation analysis revealed no significant relationship with changes in mood ($p > .75$). Further regression analysis showed that the OT-related improvements in peer attachment remained significant after correction for changes in tension ($F(1,37) = 5.00$; $p < .05$), and tentatively after correction for changes in anger ($F(1,37) = 3.5$; $p = .07$).

3.2. Secondary outcome measures: Social responsiveness and overall quality of life

3.2.1. Social Responsiveness: Social Responsiveness Scale (SRS)

After two weeks of nasal spray administration, participants of both treatment groups reported lower total SRS-scores (increased social responsiveness) (main effect of time: with outliers: $F(1,38) = 17.94$, $p < .001$, without outliers: $F(1,36)$, $p < .001$). Although tentatively more pronounced in the OT group (with outliers: $F(1,38) = 14.42$; $p < .001$; without outliers: $F(1,36) = 23.44$, $p < .001$) compared to the PL group ($F(1,38) = 4.80$; $p = .034$), the difference in slopes was not significantly different, indicating that the effect was not specific to the OT-treatment (group x time: $F(1,38) = 1.30$, $p = .26$; $\eta^2 = .03$; power = .20) (Figure 3A and Table 1) (note however that the group x time interaction effect was marginally significant after removal of two outliers in the OT group: $F(1,36) = 3.51$, $p = .07$, $\eta^2 = .09$, power = .45). Total SRS scores at baseline were not significantly different between treatment groups ($F(1,38) = .40$, $p = .54$). Subsequent analysis of the subscale scores did reveal a treatment-specific effect for the subscale 'social motivation' (group x time: $F(1,38) = 3.85$, $p = .05$; $\eta^2 = .03$; power = .20), indicating an increase in self-reported social motivation in the OT group ($F(1,38) = 16.51$, $p < .001$), not in the PL group ($F(1,38) = 1.66$, $p = .21$) (Cohen's d = .60, medium effect) (Table 1). No significant treatment-related effects were found for the other subscales (social awareness: $F(1,38) = 1.16$, $p = .29$; $\eta^2 = .03$; power = .18) (social communication: $F(1,38) = 0.10$, $p = .75$; $\eta^2 = .003$; power = .06) (rigidity/repetitiveness: $F(1,38) = 0.09$, $p = .76$; $\eta^2 = .002$; power = .06). Also no significant inter-correlations were found between pre-to-post changes in attachment and changes in social responsiveness (all, $p > .12$).

3.2.2. Quality of Life: World Health Organization Quality of Life Questionnaire (WHOQOL)

As shown in Figure 3B, a main effect of 'treatment' indicated that quality of life scores were overall higher in the PL group, compared to the OT group ($F(1,38) = 4.75$; $p < .05$) (both at baseline and post-treatment). In addition, a significant main effect of 'time' was revealed, indicating that after two weeks of nasal spray administration, both treatment groups reported an improvement in quality of life (main effect of time: $F(1,38) = 3.90$, $p = .05$). No significant

'group x time' interaction was revealed, indicating that the reported improvements were not specific to the OT-treatment ($F(1,38) = .27$, $p = .60$; $\eta^2 = .007$; power = .08). Only in the OT group however, not in the PL group, changes in quality of life were positively associated with changes in reported peer attachment (IPPA) (OT: $r = .50$, $p < .05$) (PL: $r = -.16$; $p = .50$).

4. Discussion

The current study presents results on a double-blind, between-subject, randomized placebo-controlled trial assessing the effects of two weeks of daily OT administration on measures of state and trait attachment. Reductions in attachment avoidance and increases in reports of attachment toward peers were reported after two weeks of OT treatment. OT treatment was also associated with changes in mood, indicating decreases in feelings of tension and anger in the OT group, not in the placebo group. Further, at the end of the two-week trial, both treatment groups (OT, PL) reported to experience an increase in social responsiveness and quality of life, but the effects were only specific to the OT-treatment in terms of reports on 'social motivation'.

The present study found a decrease in the experience of avoidant attachment after two weeks of OT administration as assessed using the State Adult Attachment Measure (SAAM) (Gillath et al., 2009). No significant changes in reports of secure attachment or anxious attachment were revealed. For a long time, an adult's attachment style was considered a relatively stable disposition, rooted in internal cognitive–affective working models (i.e., mental representations) of self and other, based on previous experiences in close relationships. More recently however, it has been suggested that attachment style can be transiently influenced or shaped by situational factors such as major life events or other contextual factors (Cozzarelli et al., 2003; Davila et al., 1997; Davila and Sargent, 2003; Feeney and Noller, 1992; Gillath and Shaver, 2007; Hammond and Fletcher, 1991). While notwithstanding the stability of attachment style, the SAAM questionnaire has been validated as a useful measure for capturing temporary fluctuations in the thoughts, feelings, and behaviors underlying attachment processes (Gillath et al., 2009). As a concept, *attachment anxiety* is characterized to reflect insecurity about one's own worth and abilities, extreme need for interpersonal closeness, love, and support, and worrying about being rejected or abandoned (e.g., *"I feel a strong need to be unconditionally loved right now"*). *Attachment avoidance* on the other hand, is characterized by the reluctance to trust others, an emphasis on autonomy and self-reliance, a relatively low tolerance for interpersonal intimacy and interdependence, and a tendency to down-regulate one's own emotions (e.g., *"If someone tried to get close to me, I would try to keep my distance"*). Both concepts are thought to reflect distinct dimensions of attachment style that are largely unrelated, a notion that is supported by the baseline reports of the current sample (i.e., no inter-correlation was revealed between reports of attachment anxiety or avoidance). Reports

of attachment security on the other hand, were shown to be inversely related to attachment avoidance and attachment anxiety, which is in line with the conceptualization that attachment security reflects the relative absence of anxiety and avoidance as well as a sense of faith in the responsiveness of attachment figures, and comfort with intimacy and interdependence (e.g., *"I feel like I have someone to rely on"*).

Our results indicated that two weeks of OT treatment exerted a specific influence on decreasing a person's reluctance towards closeness or trust in others (decrease attachment avoidance), but that it has no specific effect on altering a person's feelings of insecurity about one's own abilities (attachment anxiety) or one's faith in the responsiveness of attachment figures (attachment security). Overall, the finding that OT may specifically influence one's reluctance to engage in closeness or intimacy with others may be interpreted within the framework of the recently proposed affiliative-motivation hypothesis (Bartz, 2016) suggesting that OT may specifically act by increasing affiliative strivings and that individuals with a decreased tendency to affiliate (e.g. avoidantly attached individuals) may be most likely to benefit from OT treatment.

In addition to the assessments of changes in state attachment using the SAAM questionnaire, the Inventory of Parent and Peer Attachment (IPPA) questionnaire was administered prior and post OT administration to assess potential changes in trait-related conceptions of attachment towards friends and parents. Results indicated that two weeks of daily OT administration induced a medium-large enhancement in self-reported attachment towards peers, whereas no significant changes were revealed for reports of parent-oriented attachment (although non-significant tendencies were revealed for reports of attachment towards 'mother'). Note however that the observation of more pronounced effects on peer attachment, compared to parent attachment may reflect a particularity of the included sample which consisted primarily of campus-based university students with limited or no parent-contact during the two-week trial.

We explored whether changes in reports of attachment were potentially related to basic changes in mood, by additionally assessing the Profile of Mood States questionnaire (POMS) at baseline and after the two-week treatment. While previous studies on the single-dose effects of OT often report no change in mood state (Baumgartner et al., 2008; Buchheim et al., 2009; Kosfeld et al., 2005; Rimmele et al., 2009), the current results indicate that after two weeks of treatment, feelings of tension and (tentatively) anger were significantly reduced in the OT group, not in the PL group. At least for reports on the IPPA of peer attachment, it appears that inter-individual differences in (baseline) attachment style may play a pivotal role in determining treatment response. The observation that normal variance at baseline can modulate the effects of OT is in line with results from previous studies exploring the effects of a single-dose of OT. Particularly, De Dreu (2012) showed that OT administration significantly improved cooperation behavior, but only in individuals high on attachment avoidance. Similarly, a more recent study

showed that while OT produced a slight increase in communion for the average participant; avoidantly attached individuals were especially likely to perceive themselves as more communal ("kind," "warm," "gentle," etc.) after receiving OT (Bartz et al., 2015). Also in terms of social-cognitive competences, Bartz et al. (2010) showed that OT was able to improve empathic accuracy on an emotion recognition task, but only for less-socially proficient individuals (Bartz et al., 2010). While these studies and our study provide indications that OT may be particularly effective in specifically ameliorating domains that are affected in the treated individual, other lines of work provide indications that a more complex relationship might exist between variance in personality aspects and differentiated responses to OT treatment. For example, another study by (Bartz et al., 2011) showed that individuals with low scores on attachment anxiety reported more positive childhood memories after OT administration (compared to placebo), while in individuals with high scores on attachment anxiety the inverse effect was found, indicating more negative recollections on the caring behavior of their mothers after OT administration. Also positive effects of OT on one's willingness to donate money to charity (Riem et al., 2013) or prosocial behavior in a virtual ball-tossing game (van IJzendoorn et al., 2011) were shown to be lowered or absent in individuals with high reports of parental love-withdrawal. Together, these observations further highlight the importance of adequately characterizing inter-individual differences in attachment style and/or other personality traits for evaluating expectancies on OT treatment outcome.

Finally, secondary outcome measures were included to assess the effects of OT treatment on social responsiveness and quality of life using the social responsiveness scale (SRS) and the World Health Organization Quality of Life questionnaire (WHOQOL), respectively. Interestingly, participants of both treatment groups (OT and PL) reported an improvement in quality of life after two weeks of nasal spray administration which may provide relevant insights in terms of the tolerability and safety of multiple-dose OT treatment by showing no adverse events on overall wellbeing. Further, both treatment groups also reported improvements in social responsiveness (SRS) although effects were tentatively more pronounced for the OT group, compared to the placebo group. Interestingly a specific significant effect of OT was revealed for the SRS subscale assessing 'social motivation', providing further support to the notion that OT may primarily exert its effects by increasing affiliative strivings and social motivation as postulated by the affiliative-motivation hypothesis (Bartz, 2016).

To conclude, a two-week treatment with intranasally administered OT was shown to induce improvements in reports of attachment avoidance and secure peer attachment, particularly in individuals with avoidant or insecure attachment styles. The two-week treatment was well-tolerated by all participants with no reports of adverse events or impact on overall well-being. Instead, OT-specific effects were revealed for improving negative mood states and also unspecific increases in social responsiveness and quality of life were reported after

participation in the two-week trial. Although overall, these results on multiple-dose OT treatment are promising, we note that the included sample was somewhat small and restricted to males. Future work is therefore needed to be conclusive on the generalizability of the reported effects.

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Figure 1. Effects of two-week treatment on the experience of attachment. The upper graph of Panel (A) visualizes reported changes in 'peer attachment' (IPPA) for each treatment group (oxytocin (OT) – placebo (PL)) at baseline and after (post) the two-week treatment. The upper graph of Panel (B) visualizes treatment-related changes in 'attachment avoidance' (SAAM) for each treatment group (OT - PL) at baseline and post treatment. The lower graphs visualize the individual treatment responses by plotting the relationship between the baseline (pre-treatment) scores (horizontal axis) and the post-treatment scores (vertical axis). In graphical terms, the main effect of treatment is visualized by the vertical distance between the regression lines of the OT group (grey line) and the PL group (black line). The dotted line represents a perfect linear relationship (x=y) indicating no change from pre-to-post treatment.

Note that for changes in IPPA 'peer attachment' (lower panel A), the regression line of the PL group largely overlapped with the perfect linear regression line, indicating no change from pre-to-post in the PL group. Importantly, for IPPA peer attachment, a significant 'treatment group x baseline score' interaction effect was revealed, indicating a difference in slopes of the regression lines for the OT treatment group and the PL group (i.e., regression lines are not parallel). Overall, the shape of the interaction pattern indicated that the difference in outcome between treatment groups increased with increasing baseline 'severity' (lower baseline peer attachment). In other words, the OT treatment appeared to enhance IPPA peer attachment particularly among those with low peer attachment at baseline.

For changes in SAAM 'attachment avoidance' (lower panel B), no significant difference was revealed between the slopes of the regression lines of the OT and PL treatment groups, indicating that the main effect of treatment (the vertical distance between the regression lines) was constant irrespective of initial baseline score. Note however that for both groups (OT and PL), reductions in SAAM attachment avoidance were most pronounced for participants with high baseline attachment avoidance.

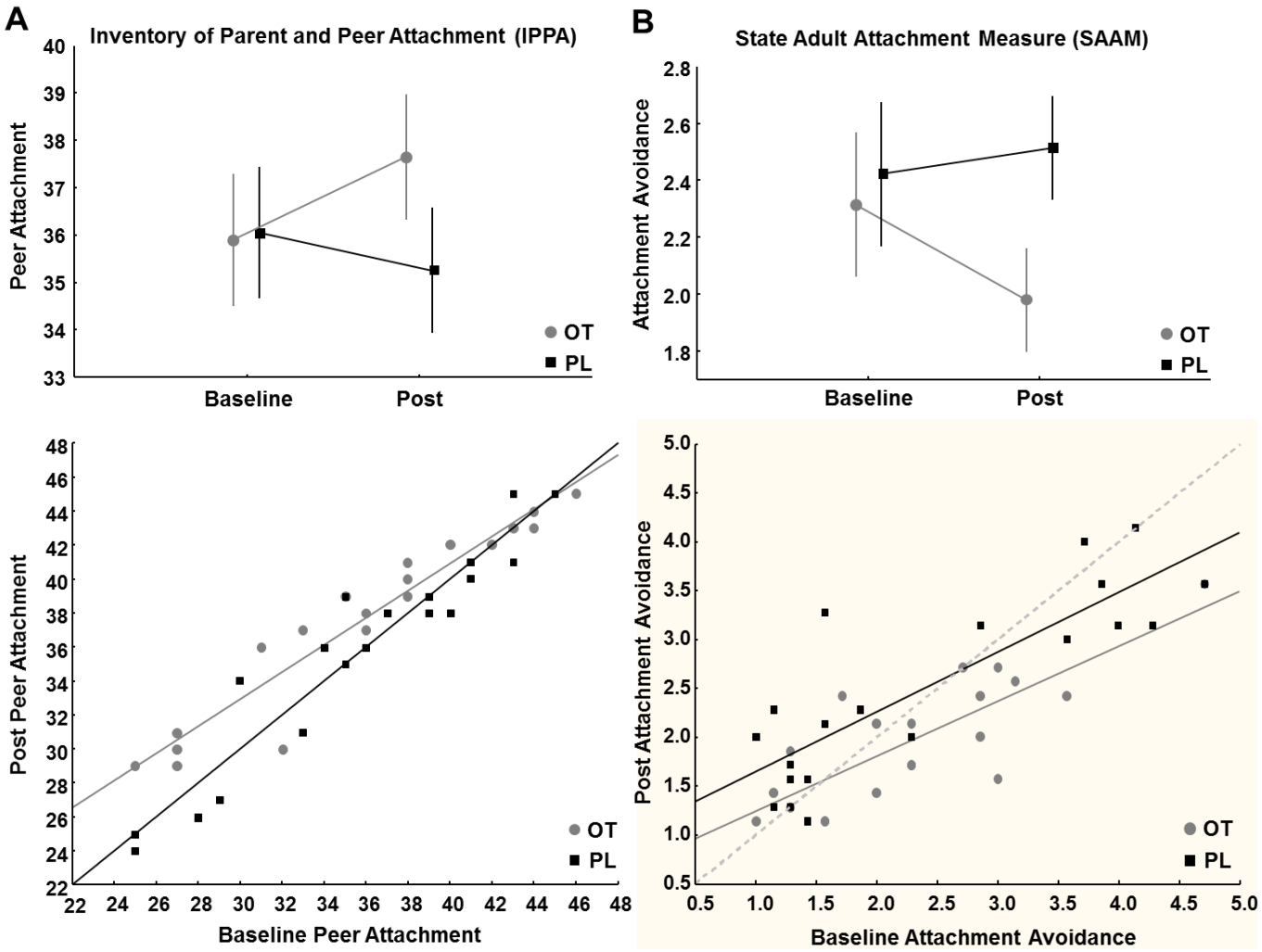

Figure 2. Effects of the two-week treatment on mood states. The upper graph visualizes treatment-related changes in feelings of 'tension' (POMS) for each treatment group (oxytocin (OT) - placebo (PL)) at baseline and after (post) the two-week treatment. The lower graph visualizes treatment-related changes in feelings of 'anger' (POMS).

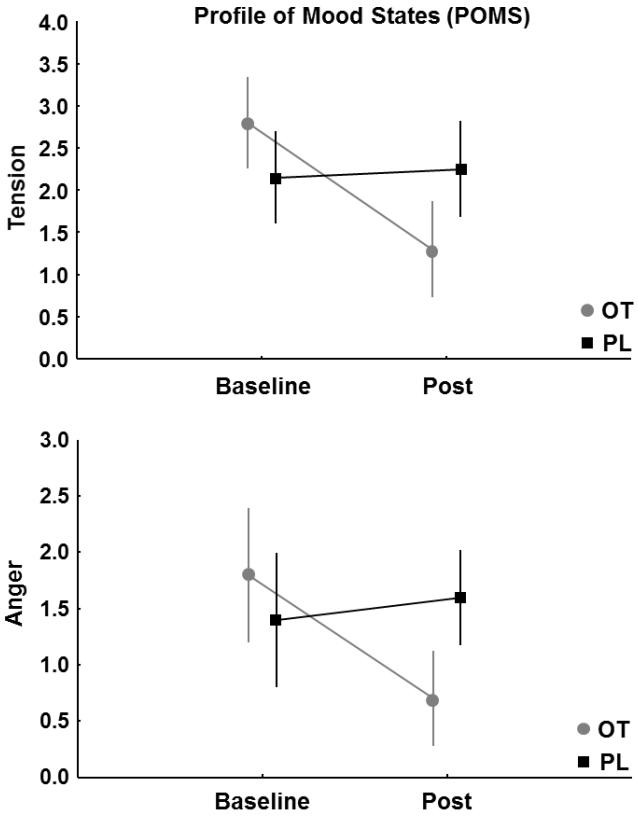

Figure 3. Effects of the two-week treatment on social responsiveness and quality of life. Panel (A) visualizes changes in 'social responsiveness' (SRS) for each treatment group (oxytocin (OT) - placebo (PL)) at baseline and after (post) the two-week treatment. Panel (B) visualizes changes in 'quality of life' (WHOQOL).

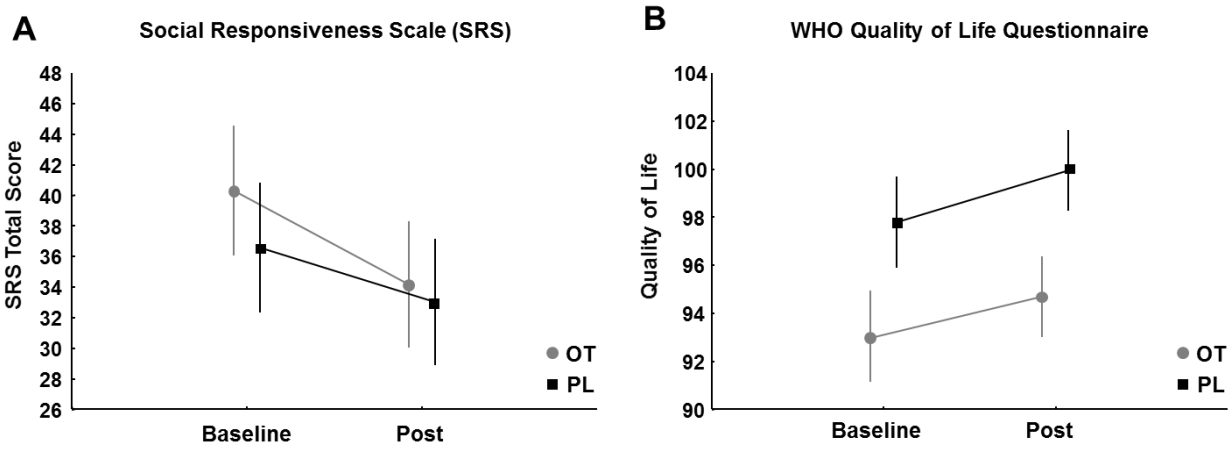

Table 1. Change from baseline scores (with outliers) for each treatment group (oxytocin, placebo) and corresponding Cohen's d effect sizes (Performance change$_{OT}$ – Performance change$_{PL}$)/pooled SD).

Outcome Measure	**Oxytocin**	**Placebo**	
	Change from baseline	**Change from baseline**	
Self-report Questionnaires	Mean ± SD	Mean ± SD	**Cohen's d**
Trait Attachment - IPPA			
Friends	1.75 ± 1.97	-0.80 ± 4.02	0.75
Mother	0.65 ± 1.98	-0.20 ± 1.85	0.44
Father	0.90 ± 1.77	-0.35 ± 4.73	0.35
Important person	-0.09 ± 2.12	0.19 ± 2.54	0.10
State Attachment - SAAM			
Security	0.08 ± 0.40	0.18 ± 0.49	0.22
Anxiety	-0.17 ± 0.72	-0.18 ± 0.68	0.01
Avoidance	-0.34 ± 0.56	0.09 ± 0.72	0.63
Mood - POMS			
Tension	-1.50 ± 1.88	0.10 ± 2.90	0.63
Depression	-0.35 ± 1.23	0.60 ± 3.42	0.37
Anger	-1.10 ± 2.29	0.20 ± 1.58	0.63
Vigor	-0.55 ± 4.42	0.00 ± 4.58	0.12
Fatigue	-0.85 ± 3.25	-1.45 ± 4.83	0.15
Social responsiveness - SRS-Adult			
Social Awareness	-1.45 ± 2.74	-0.30 ± 3.91	0.34
Social Communication	-1.20 ± 3.05	-0.90 ± 2.90	0.10
Social Motivation	-2.05 ± 1.93	-0.65 ± 2.54	0.60
Rigidity and Repetitive behavior	-1.45 ± 2.16	-1.70 ± 2.92	0.10
Total	-6.15 ± 5.93	-3.55 ± 8.35	0.35
Quality of life - WHOQOL-Bref			
Total	1.25 ± 4.54	2.15 ± 6.23	0.17

Note. SAAM = State Adult Attachment Scale; IPPA = Inventory of Parent and Peer Attachment; POMS = Profile of Mood States; SRS-Adult = Social Responsiveness Scale, adult version; WHOQOL-bref = World Health Organization Quality of Life, abbreviated version.